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(54) Title: PHARMACEUTICAL COMPOSITIONS CONTAINING AROMATIC POLYMERS AND THERAPEUTIC METHODS USING THE SAME (57) Abstract The present invention describes novel pharmaceutical compositions containing polyaromatic compounds having properties which mimic pharmacological activities of glycosaminoglycans which effect the distribution in tissue of biologically active peptides and proteins normally bound to glycosaminoglycans and to methods of therapy using said pharmaceutical compositions.		

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PHARMACEUTICAL COMPOSITIONS CONTAINING AROMATIC
POLYMERS AND THERAPEUTIC METHODS USING THE SAME

Field of Invention

The present invention relates to methods of therapy involving the use of said pharmaceutical compositions containing polyaromatic compounds having properties which mimic pharmacological activities of glycosaminoglycans and effect the distribution in tissue of biologically active peptides and proteins normally bound to glycosaminoglycans.

Glycosaminoglycans (GAG) are linear polysaccharides formed by characteristic repeating disaccharide units usually composed of a uronic acid and a hexosamine. The term "acid mucopolysaccharides" was used originally to designate hexosamine-rich acid polysaccharides extracted from connective tissue. In recent years, the term "glycosaminoglycans" has gained greater acceptance and is now used in place of mucopolysaccharides. The hexosamine can be glycyamine or galactosamine, and the uronic acid can be glucuronic or iduronic acid. Sulphate groups are found on all glycosaminoglycans apart from hyaluronic acid, and all of the sulphated

glycosaminoglycans are covalently linked to protein forming different classes of proteoglycans. However, it would be an oversimplification to consider glycosaminoglycans to be simple repeat-unit polysaccharides, since considerable chemical and configurational variability can be superimposed upon the component sugars.

Among other functions it has been shown that the glycosaminoglycans serve also as a support which binds various bioactive peptides. This association is based on a non-covalent interaction since the bound protein can be readily released upon the addition of free glycosaminoglycans. Well known examples of such bound proteins include enzymes such as lipoprotein lipase (LPL) or growth-regulating peptides such as fibroblast growth factor (FGF). Another example of GAG-protein interaction is that of the enzyme heparinase which participates in cell-invasion processes. It has been demonstrated also that the commercially available glycosaminoglycan, heparin, inhibits the growth of vascular smooth muscle cells and the proliferation of kidney mesangial cells. The former cell type is involved in atherosclerosis while the latter plays a role in glomerulosclerosis.

Heparin is known also to be involved in the release of lipoprotein lipase, the inhibition of heparinase and the release of fibroblast growth factor. The most common application of heparin is as an anticoagulant where heparin interacts with proteins which play a key role in hemostasis.

Glycosaminoglycans such as heparin are a major constituent participating in the composition of various biological structures such as basement membranes, connective tissues, cartilage and cell-surface glycocalyx. Connective tissues are responsible for providing and maintaining form in the body. Functioning in a mechanical role, they provide a matrix that serves to connect and bind the cells and organs and ultimately give support to the body. Unlike the other tissue types (epithelium, muscle and nerve) formed mainly by cells, the major constituent of connective tissue is its extracellular matrix, composed of protein fibers, an amorphous ground substance, and tissue fluid, the latter consisting primarily of bound water of solvation. Embedded within the extracellular matrix are the connective tissue cells.

In terms of structural composition, connective tissue can be subdivided into three classes of components: cells, fibers and ground substance. The wide variety of connective tissue types in the body represents modulations in the degree of expression of these three components.

The amorphous intercellular ground substance fills the space between cells and fibers of the connective tissue; it is viscous and acts as a lubricant and also as a barrier to the penetration of the tissues by foreign particles. Glycosaminoglycans and structural glycoproteins are the two principal classes of components comprising the ground substance.

The present invention is based on the discovery of a class of compounds exhibiting properties which mimic

the action of glycosaminoglycans and which are capable of modulating biological systems containing complexes between bioactive peptides and/or proteins and glycosaminoglycans by competing with the binding interactions of glycosaminoglycans.

Summary of the Invention

The present invention relates to a pharmaceutical composition comprising, in admixture with a pharmaceutically acceptable carrier, a therapeutically effective amount of a polyaromatic ring-containing polymeric compound, substantially free of monomer, and having properties which mimic the pharmacological activity of glycosaminoglycans and which are capable of competing with the binding thereof to bioactive peptides and/or proteins.

The present invention relates more specifically to a pharmaceutical composition including as an active ingredient a therapeutically effective amount of a polyaromatic ring-containing polymeric compound having a molecular weight between about 2,000 and about 20,000 Daltons wherein each monomeric unit of said polymer includes from 1 to about 10 aromatic rings, which may be substituted by electronegative substituents and/or negatively charged residues.

This invention relates also to therapeutic methods comprising the administration of an effective amount of the above-mentioned pharmaceutical composition to human or other animal patients in need of cardiovascular therapy such as anticoagulant and/or antithrombotic

therapy and/or bone metabolic therapy and/or therapy for the treatment of neuronal disorders.

Detailed Description

As employed above and throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

"Distribution in tissue" or "tissue distribution" is used herein to mean either the compartmentalization of a molecular constituent within a given tissue or the distribution pattern between different tissues.

"Tissue redistribution" means a change in the compartmentalization within a given tissue or a change in the distribution pattern between tissues. For example, a peptide may be released from a basement membrane which can result subsequently in its consumption by surrounding cells or its transfer into the bloodstream, or a soluble compound could be bound to a protein in a competitive binding situation thereby preventing the association of said compound with certain tissue-fixed residues.

"Alkyl" means a saturated aliphatic hydrocarbon which may be either straight or branched-chained containing from about 1 to about 12 carbon atoms.

"Lower alkyl" means an alkyl group as above, having 1 to about 4 carbon atoms.

"Substituted phenyl" means a phenyl group substituted with one or more substituents which may be alkyl, alkoxy, amino, nitro, carboxy, carboalkoxy, cyano, alkyl amino, halo, hydroxy, hydroxyalkyl, mercaptyl, alkyl mercaptyl, carboalkyl or carbamoyl.

Certain of the polymeric compounds included in the compositions of the present invention may exist in enolic or tautomeric forms, and all of these forms are considered to be included within the scope of this invention.

The polymeric compounds included in the compositions of this invention may be useful in the form of the free base, in the form of salts, esters and as hydrates. All forms are within the scope of the invention. Acid addition salts may be formed and are simply a more convenient form for use; and in practice, use of the salt form inherently amounts to use of the base form. The acids which can be used to prepare the acid addition salts include preferably those which produce, when combined with the free base, pharmaceutically acceptable salts, that is, salts whose anions are non-toxic to the animal organism in pharmaceutical doses of the salts, so that the beneficial pharmacological properties inherent in the free base are not vitiated by side effects ascribable to the anions. Although pharmaceutically acceptable salts of said basic compound are preferred, all acid addition salts are useful as sources of the free base form even if the particular salt per se is desired only as an intermediate product as, for example, when the salt is formed only for purposes of purification and identification, or when it is used as an intermediate in preparing a pharmaceutically acceptable salt by ion exchange procedures. Pharmaceutically acceptable salts of the compounds useful in the practice of this invention are those derived from the following acids: mineral acids such as hydrochloric acid, sulfuric acid, phosphoric acid and sulfamic acid; and organic acids such as acetic acid, citric acid, lactic acid, tartaric

acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, quinic acid, and the like. The corresponding acid addition salts comprise the following: hydrochloride, sulfate, phosphate, sulfamate, acetate, citrate, lactate, tartarate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate and quinate, respectively.

The acid addition salts of the compounds contained in the compositions of the present invention are prepared either by dissolving the free base in aqueous or aqueous-alcohol solution or other suitable solvents containing the appropriate acid and isolating the salt by evaporating the solution, or by reacting the free base and acid in an organic solvent, in which case the salt separates directly or can be obtained by concentration of the solution.

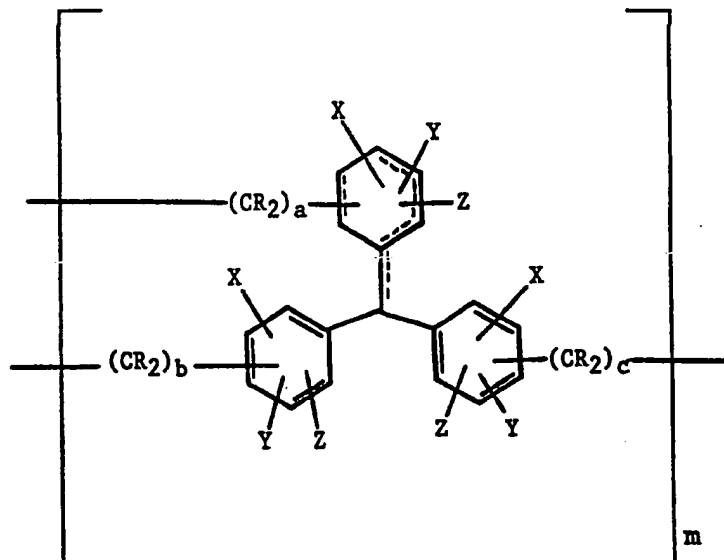
Preferred pharmaceutical compositions include polymeric compounds having monomeric units containing about three to about ten aromatic rings and having, on at least two of the aromatic rings, one or more substituent selected from the group consisting of NRR_1 , $-\text{N}=\text{R}$, $-\text{OR}$, $=\text{O}$, $-\text{NO}_2$, $-\text{COOR}$, halogen, $-\text{SO}_2\text{OR}$, $-\text{SO}_2\text{NHR}$, $-\text{OSO}_2\text{OR}$ and R , wherein R is C_1 - C_{12} alkyl or hydrogen and R_1 is lower alkyl, hydrogen, phenyl or substituted phenyl.

The more preferred number of aromatic rings in each monomeric unit is about three to about six.

Polymers of many known synthetic dyes comprising monomers having the polyaromatic characteristics identified above are within the scope of the present invention and are useful in pharmaceutical compositions. Such dyes are known to be useful as color additives, diagnostic aids administered by i.v. injection, antiseptic agents and/or to treat infectious disease. Nevertheless, none of these dyes has previously been described as useful as or used as a therapeutic agent as a replacement for glycosaminoglycans.

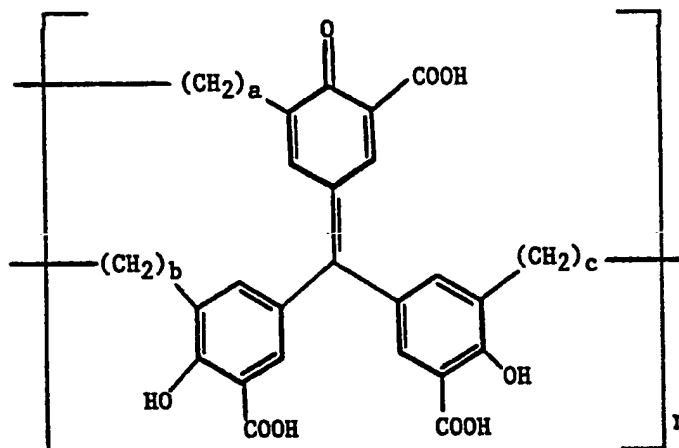
Exemplary synthetic dyes including polymeric materials in accordance with this invention include Aluminon, Halogenated Aluminon, Sulfonated Aluminon, Sulfonated-Halogenated Aluminon, Anazoline Sodium, Eosine I Bluish, Eosine Yellowish, Erythrosine, Evan's Blue, Fast Green FCF, Fuchsin(e) Acid, Iodophthalein Sodium, Rose Bengal, Sulfobromophthalein Sodium, Suramin Sodium, Trypan Blue, Trypan Red, Rosaniline Chloride, Crystal Violet, Methyl Blue, Methyl Green, Coomassie Blue, Basic Fuchsin, Malachite Green, Brilliant Green, Aniline Blue, Brilliant Cresyl Blue, Safranin O, Ethyl Violet, Pararosaniline Acetate and Methyl Violet.

More preferred is a polymeric compound of the structure:



or a salt or ester thereof, wherein a , b and c are independently 0 or 1 and m is about 5 to about 20, and dashed lines represent single or double bonds, each aromatic ring is substituted with at least one substituent (x , y , z) selected from $-NRR_1$, $-N=R$, $-OR$, $=O$, $-NO_2$, $-COOR$, halogen, $-SO_2OR$, SO_2NHR , $-OSO_2OR$ and R , wherein R is lower alkyl or hydrogen and R_1 is lower alkyl, hydrogen or phenyl and substituted phenyl.

Most preferred is a polymeric compound of the structure:



wherein a, b and c are independently 0 or 1 and $a+b+c \geq 2$ and m is about 5 to about 20.

The preferred molecular weight of the polymeric compounds is about 2,000 and about 20,000, and the most preferred molecular weight is about 2,000 to about 4,000 Daltons, as measured by gel permeation chromatography.

The following gel permeation chromatography conditions has been used to measure the molecular weight of the polymeric compounds contained in the product identified commercially, and in the Merck Index, as aurintricarboxylic acid (aluminon or ATA).

Column: Two I-125 (Waters, TN) protein analysis column in series

Mobil Phase: Water (70%)-propanol (30%)
containing 0.1% (V/V) trifluoroacetic acid

Detection: UV at 220m

Injection: Sample was dissolved in the mobile phase and injected

Pharmaceutical compositions containing the pharmacologically active polymeric compounds are believed to function according to one or more of the following mechanisms.

1. Removal of cationic proteins from the glomerular basement membrane or connective-tissues, thereby preventing local damage (i.e., via "recharging" of negatively charge residues in the glomerular basement membrane).
2. Modulation of LPL, a key enzyme in lipid distribution among various tissues which could be implicated in cardiovascular diseases.
3. Release of growth-promoting molecules, such as fibroblast growth factor (FGF), from basement membranes in order to enhance the process of angiogenesis and wound-healing. In addition, FGF can prevent death of lesioned neurons (see Anderson K.J. et al. Nature 332:360-1 (1988)). By the release of endogenously-stored FGF, the compositions within the scope of the present invention may be useful in the treatment of neuronal disorders such as Alzheimer's disease and other dementia.
4. Blocking the activity of heparinase, an enzyme which participates in inflammatory processes and metastases formation.
5. Modulation of bone metabolism.
6. Control of the proliferation of certain cell types such as smooth muscle cells or mesangial cells.

Preferred embodiments of the present invention are described in the following non-limiting examples which

include a description of pharmacological test procedures believed to correlate to therapeutic activity in humans and other animals.

EXAMPLE 1

It has been determined that the pharmaceutical activity of the mixture of compounds commercially available as "Aluminon" (aurintricarboxylic acid or ATA) is produced by the fraction of the mixture containing polymers having a molecular weight of about 2,000 to about 20,000 Daltons. These polymeric materials are believed to comprise monomeric units of ATA.

The following comparative work shows the relative pharmacological activities of various fractions of the commercial ATA product. On the basis of the following work compositions within the scope of the present invention exhibit useful anticoagulant and antithrombotic activity.

Experimental Procedures

Comparative Compound A - Commercially available aurointricarboxylic acid (ATA) from Aldrich Chemical Co., Wisconsin.

Compound B - Polymer containing ATA monomer and having molecular weight of about 2,000 to about 4,000 Daltons.

This material is prepared using commercially available aurointricarboxylic acid which is fractionated on Biogel P-6 by elution with 50% aqueous ethanol with 50mM Tris at pH 7.5 and then 50% aqueous ethanol with 50mM glycine at pH 11. The fraction containing polymeric

material having a molecular weight of about 2000 to about 4000 Daltons is separated.

Comparative Compound C - ATA not containing polymers. Following the procedure described in Organic Synthesis, Coll. Vol. I, page 54, the following reaction was performed to produce what is believed to comprise non-polymerized ATA. To 17 ml of concentrated sulfuric acid at 0-5°C and mechanically stirred was added portionwise sodium nitrite (2.5 gm, 36.2 mmol). The mixture was stirred until a solution was obtained. Salicylic acid (5.0 gm, 36.5 mmol) was added portionwise over 10-15 minutes and the reaction warmed to room temperature, stirred until a homogeneous mixture was obtained and cooled to 0-5°C. A solution of formaldehyde (formalin, 35-40%, 1.25 mL, 16.3 mmol) was added dropwise over 20 minutes. The reaction was quenched with the slow addition of 25 gm crushed ice and then 125 mL of ice water. The resulting solid was filtered, washed with water and dissolved in dilute aqueous ammonium hydroxide. Removal of the volatiles at 90°C and then in vacuo provided the red-brown solid.

Comparative Compound D - Polymer believed to contain ATA monomeric units is prepared by using a large excess of formalin in the above procedure along with the following reagents.

concentrated sulfuric acid: 17mL
sodium nitrite: 2.5 gm, 36.2 mmol
salicylic acid: 5.0 gm, 36.5 mmol
formalin: 35-40%, 6.0 mL, 81.5 mmol

The pharmacological activity of Compounds A through D is measured according to the following test procedure.

Activated Partial Thromboplastin Time (APTT)

To an assay cuvette is added 100 ul of normal pooled plasma (George King Biomedical Inc., Kansas) and 100 ul of a solution containing one of the above compounds in aqueous 50 mM Tris hydrochloride at pH 7.5 (0.2 mg of sample in one mL buffer). The sample is placed in a MLA coagulation timer which automatically maintains the sample at 37°C for 2.5 minutes, 100 ul of actin activated cephaloplastin reagent is injected, kept 5 minutes, 100 ul of 35 mM CaCl is injected, and clot formation is determined photometrically and the time recorded. Results are presented in TABLE I below.

TABLE I

<u>Compound</u>	<u>Concentration (mg/mL)</u>	<u>APTT (sec.)</u>
Normal pooled plasma	-----	25 - 28
A - Commercial ATA	0.2	40 - 60
C - Non-Polymerized ATA	0.2	28
D - Polymer containing ATA monomer	0.2	72
B - Polymer having molecular weight of 2,000 to 4,000 containing ATA monomer	0.2	110

The above comparative test results demonstrate that a polymer having a molecular weight of 2000 to 4000 Daltons and believed to contain monomers of ATA exhibit anticoagulant activity.

All of the xamples pres nted below involv the use of commercially available mat rials. Based on the

above example, the polymeric components of the commercially available products are believed to be the source of the APTT activity discovered in the commercial products.

EXAMPLE 2

The polymeric components of the following dyes, at the concentration listed, significantly prolong the activated partial thromboplastin time when compared to a standard buffer solution.

<u>Compound</u>	<u>Minimum Final Concentration (ug/ml)</u>
Rosaniline Chloride	12.5
Crystal Violet	8.3
Methyl Blue	100.0
Methyl Green	50.0
Coomassie Blue	150.0
*Basic Fuchsin	50.0
Malachite Green	150.0
Brilliant Green	150.0
Aniline Blue	150.0
Brilliant Cresyl Blue	150.0
Safranin O	150.0
Ethyl Violet	50.0
Pararosaniline Acetate	150.0
Methyl Violet	75.0

EXAMPLE 3

The effect on enzyme levels and enzyme compartmentalization of compositions within the scope of the present invention are measured according to the test procedure described below.

Modulation of Lipoprotein Lipase (LPL) Levels and Compartmentalization

The enzyme LPL participate in the process of lipid transfer from the bloodstream to the tissues, LPL is bound to the external surface of cells via its association with cell-membrane glycosaminoglycans. Cultured heart cells is a powerful system in the study of the enzyme turn-over and the exploration of ways to manipulate its level since these cells are active in the biosynthesis of LPL, in addition to the surface binding of the enzyme.

The following are the materials and methods used:

F₁ heart cell cultures are prepared from newborn rate hearts as previously described (Chajek, T. et al., Biochem. Biophys. Acta 528, 456-474 (1978)). They consist mainly of non-beating mesenchymal cells and are used 8-12 days after their isolation.

Enzyme activity is determined using aliquots of medium and of homogenates of cells which are released from a petri dish with a rubber policeman in 1 ml of 0.025 M NH₃/NH₄Cl buffer (pH 8.1) containing 1 unit/ml of heparin. The assay system consists of 0.1 ml of medium or 0.1 ml of cell homogenate (50-70 g protein) and 0.1 ml of substrate containing labeled triolein, prepared according to the method of Nilsson-Ehle and Schotz, (J. Lipid Res. 17, 536-541 (1976)). Incubations are carried out at 37°C for 45 mins. The reaction is stopped by the addition of methanol/chloroform/heptane (1.4:1.25:1.v/v) and the extraction of fatty acids is performed according to the method of Belfrag and Vaughan (J. Lipid Res. 10, 341-344 (1969), as modified by Nilsson-Ehle and Schotz.

Enzyme activity is calculated according to the formula of Nilsson-Ehle and Schotz.

Table II below demonstrates that commercially available Evan's Blue modulates or shifts-up enzyme levels and influences enzyme compartmentalization.

TABLE II

LPL activity following 24 hrs. incubation of heart cells with commercially available Evan's Blue
LPL activity: m mole FFA/h/dish
(mean \pm SEM of triplicates)

Evan's Blue Concentration (M)	Cells	Medium	Total	% increase in the total
0	2420 \pm 93	817 \pm 14	3237	-(0)
10 ⁻⁵	2757 \pm 114	1531 \pm 182	4288	+32.5
10 ⁻⁴	2447 \pm 48	2783 \pm 194	5230	+61.6
10 ⁻³	1698 \pm 184	6051 \pm 435	7749	+139.4

Similar results are obtained with commercially available Methyl Blue, Trypan Blue and Aluminon. The foregoing results indicate that compositions within the scope of this invention are useful in the treatment of cardiovascular diseases such as arteriosclerosis.

EXAMPLE 4

The active ingredients in the composition of the present invention are effective inhibitors of heparinase activity. The inhibitory activity is measured by the following procedure.

Inhibition of Heparinase Activity

Heparinase is an endoglucuronidase capable of degrading heparin sulfate (HS) at specific intrachain sites. Studies on degradation of sulfated proteoglycans in the subendothelial extracellular matrix (ECM) demonstrated a correlation between heparinase activity and the metastatic potential of various tumor cells. Heparinase activity was also suggested to play a role in the mobilization of normal circulating cells of the immune system during inflammatory processes.

The ability of compositions of the present invention to inhibit lymphoma-cell derived heparinase is tested in the assay system described by Vlodavsky et al. (Cancer Research. 43:2704-2711 (1983)). ³⁵S labeled ECM is incubated for 24 hours with ESb mouse lymphoma heparinase in the presence of 10 ugr ml of the indicated compounds. Degradation of the sulfated glycosaminoglycan is followed by gel filtration of the supernatants. Heparinase activity is expressed as the total amount of labeled low-molecular-weight fragments released from the EMC substrate.

Exemplary compositions are tested for inhibitory activity are the results of this test work is summarized in Table III below.

TABLE III

Inhibition of heparinase activity by various
commercially available compounds

Compound (10 μ gr/ml)	Total low-mol-wt. HS fragments released by heparinase (cpm)	% Inhibition
Control	17,756	--
Methyl Blue	13,676	23
Fast Green	12,139	32
Evan's Blue	6,986	60
Aluminon	3,822	78

Anticoagulant properties are evaluated in
accordance with the following method.

EXAMPLE 5

The anticoagulant effect of commercially available Aluminon is demonstrated by measuring the partial thromboplastin time (PTT) in blood samples from a normal donor. 0.25 ml of the indicated concentration of the drug (x10, diluted in saline) is added to 2.25 ml of fresh human blood, collected in the presence of citrate. PTT is measured following the addition of Ca^{++} .

The results are summarized in Table IV below.

TABLE IV

PTT of human blood in the presence of various concentrations of commercially available Aluminon

<u>Aluminon final conc. (mg/ml)</u>	<u>PTT (sec.)</u>
1	>300
0.1	190
0.01	30.5
0 (control)	30.5

Commercially available aluminon is an effective anticoagulant. Studies in laboratory animals, such as laboratory rats, demonstrate that aluminon can be absorbed through the gastrointestinal tract. Oral administration of commercially available aluminon to rats (1 ml of 10-20% solution) prolongs rats' PTT considerably (from around 20 sec. at time 0 to >200 sec. after 2-4 hours); concomitantly, plasma levels of LPL were elevated by about an order of magnitude. Consequently, the compositions of the present invention are capable of being administered orally to produce an anticoagulant effect. In contrast, heparin can only be administered by injection.

EXAMPLE 6

The active ingredients of the compositions within the scope of this invention are useful inhibitors of the proliferation of vascular smooth muscle cells. The proliferation inhibition of the active ingredients of the present compositions may be evaluated according to the following procedure.

Inhibition of Vascular-Smooth Muscle Cell Proliferation

Bovine aortal smooth-muscle cells are grown in tissue culture plates in the presence of glucose-rich medium (H-21). The cultures (around 35×10^3 cells/well in 24 wells' plates) are initially supplemented with 10% fetal calf serum (FCS) for 2-3 days. The cell nutrition is then replaced by a serum-deprived medium (H-21 plus 0.2% FCS) in order to test the effect of defined growth factors on the proliferation of smooth-muscle cells and their possible inhibition by the compounds with heparin-like activity.

In the following representative experiment, the cells transferred to the serum-deprived medium are grown in the presence of thrombin (10^{-6} M) or FGF (fibroblast growth factor, partially purified from bovine brain, 250 ngr/ml). Various concentrations of Aluminon were added to the thrombin or FGF-treated cells and to non-treated control cultures. ^3H thymidine incorporated into DNA was measured. The results are presented in Table V below.

TABLE V

Growth inhibition of resting, FGF or thrombin-stimulated smooth muscle cells by commercially available aluminon

Aluminon Concentration ($\mu\text{gr/ml}$)	H ³ thymidine incorporation per well (cpm. mean of duplicates)		
	Control	+FGF	+Thrombin
0	9,823	22,574	54,000
0.2	8,425	22,256	46,857
2	5,120	13,089	14,064
10	4,105	6,846	6,877
20	4,343	6,745	5,705

The results of Table V show that Aluminon, at a concentration of 2 $\mu\text{gr/ml}$ ($\sim 4 \times 10^{-6}\text{M}$), is effective in inhibiting labeled thymidine incorporation by more than 40% and is capable of mimicking the action of heparin in this system as a potent inhibitor of the proliferation of vascular smooth muscle cells.

Compositions of the present invention are useful in the treatment of cardiovascular diseases, including arteriosclerosis, bone metabolism and neuronal disorders.

The compositions of this invention can be normally administered orally or parenterally, in the treatment of cardiovascular disorders, bone metabolic disorders and neuronal disorders in humans or other mammals.

The compositions of this invention may be formulated for administration in any convenient way, and the invention includes within its scope pharmaceutical compositions containing at least one polymeric compound as described hereinabove adapted for use in human or veterinary medicine. Such compositions may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers or excipients. Suitable carriers include diluents or fillers, sterile aqueous media and various non-toxic organic solvents. The compositions may be formulated in the form of tablets, capsules, lozenges, troches, hard candies, powders, aqueous suspensions, or solutions, injectable solutions, elixirs, syrups and the like and may contain one or more agents selected from the group including sweetening agents, flavoring

agents, coloring agents and preserving agents, in order to provide a pharmaceutically acceptable preparation.

The particular carrier and the ratio of therapeutically effective compound to carrier are determined by the solubility and chemical properties of the compounds, the particular mode of administration and standard pharmaceutical practice. For example, excipients such as lactose, sodium citrate, calcium carbonate and dicalcium phosphate and various disintegrants such as starch, alginic acid and certain complex silicates, together with lubricating agents such as magnesium stearate, sodium lauryl; sodium lauryl sulphate and talc, can be used in producing tablets. For a capsule form, lactose and high molecular weight polyethylene glycols are among the preferred pharmaceutically acceptable carriers. Where aqueous suspensions for oral use are formulated, the carrier can be emulsifying or suspending agents. Diluents such as ethanol, propylene glycol, glycerin and chloroform and their combinations can be employed as well as other materials.

For parenteral administration such as intramuscular and subcutaneous injection, solutions or suspensions of the polymeric compounds in sesame or peanut oil or aqueous propylene glycol solutions, as well as sterile aqueous solutions can be employed. The aqueous solutions using pure distilled water are also useful for intravenous injection purposes, provided that their pH is properly adjusted, suitably buffered, made isotonic with sufficient saline or glucose and sterilized by heating or by microfiltration.

The dosage regimen in carrying out the methods of this invention is that which insures maximum therapeutic response until improvement is obtained and thereafter the minimum effective level which gives relief. Thus, in general, the dosages are those that are therapeutically effective in increasing the clotting time of blood, decreasing the chances of thrombosis, reducing the buildup of arterial plaque or in the treatment of bone metabolic or neuronal disorders such as Alzheimer's disease. In general, the oral dose may be between about 1 mg/kg and about 500 mg/kg (preferably in the range of 1 to 10 mg/kg), and the i.v. dose about 0.1 mg/kg to about 10 mg/kg (preferably in the range of about 0.1 to about 3 mg/kg), bearing in mind, of course, that in selecting the appropriate dosage in any specific case, consideration must be given to the patient's weight, general health, age and other factors which may influence response to the drug. The drug may be administered as frequently as is necessary to achieve and sustain the desired therapeutic response. Some patients may respond quickly to a relatively large or small dose and require little or no maintenance dosage. On the other hand, other patients may require sustained dosing from about 1 to about 4 times a day depending on the physiological needs of the particular patient. Usually the drug may be administered orally 1 to 4 times per day. It is anticipated that many patients will require no more than about one to about two doses daily.

It is also anticipated that the present invention would be useful as an injectable dosage form which may be administered in an emergency to a patient suffering

from stroke or heart attack. Such treatment may be followed by intravenous infusion of the active polymeric compound and the amount of compound infused into such a patient should be effective to achieve and maintain the desired therapeutic response.

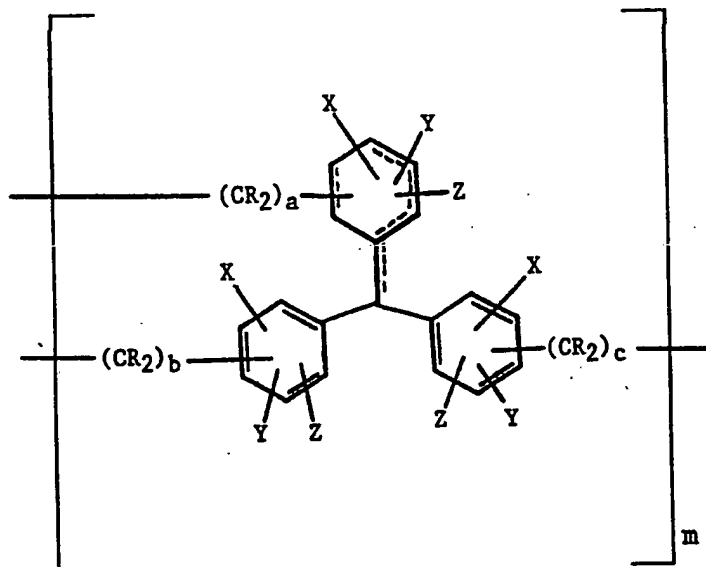
It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is therefore desired that the present embodiments and examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising, in admixture with a pharmaceutically acceptable carrier, a therapeutically effective amount of a polyaromatic ring-containing polymeric compound, substantially free of monomer, and having properties which mimic the pharmacological activity of glycosaminoglycans and which are capable of competing with the binding thereof to bioactive peptides and/or proteins.
2. A pharmaceutical composition according to Claim 1 wherein said polyaromatic ring-containing polymeric compound has a molecular weight of about 2,000 to about 20,000 Daltons and wherein each monomeric unit thereof includes from 1 to about 10 aromatic rings, which may be substituted by electronegative substituents and/or negatively charged residues.
3. A pharmaceutical composition according to Claim 2 wherein each monomeric unit contains between 3 and 10 aromatic rings.
4. A pharmaceutical composition according to Claim 3 wherein said aromatic rings contain at least one substituent on at least two of the rings.
5. A pharmaceutical composition of Claim 4 wherein the substituents are selected from $-NRR_1$, $-N=R$, $-OR$, $=O$, $-NO_2$, $-COOR$, halogen, $-SO_2OR$, $-SO_2NHR$, $-OSO_2OR$ and $-R$, wherein R is C1-C12 alkyl or hydrogen and R_1 is lower alkyl, hydrogen, phenyl and substituted phenyl.

6. A pharmaceutical composition according to Claim 1 wherein said polymeric compound has anticoagulant properties.
7. A pharmaceutical composition according to Claim 1 capable of being administered orally.
8. A pharmaceutical composition according to Claim 1 wherein said polymeric compound is capable of being absorbed into the bloodstream from the gastrointestinal tract.
9. A pharmaceutical composition according to Claim 2 wherein said monomer comprises about 3 to about 6 aromatic rings.
10. A pharmaceutical composition according to Claim 2 wherein said polymer contains monomer units selected from Aluminon, Anazoline Sodium, Eosine I Bluish, Eosine Yellowish, Erythrosine, Evan's Blue, Fast Green FCF, Fuchsin(e) Acid, Iodophthalein Sodium, Methyl Blue, Rose Bengal, Sulfobromophthalein Sodium, Suramin Sodium, Trypan Blue, Trypan Red, Rosaniline Chloride, Crystal Violet, Methyl Green, Coomassie Blue, Basic Fuchsin, Malachite Green, Brilliant Green, Aniline Blue, Brilliant Cresyl Blue, Safranin O, Ethyl Violet, Pararosaniline Acetate and Methyl Violet.

11. A pharmaceutical composition according to Claim 1 wherein said polymer is of the formula



wherein: a, b and c are independently 0 or 1;

m is about 5 to about 20;

dashed lines represent single or double bonds;

each aromatic ring is substituted with at

least one substituent (x, y, z) selected

from $-NRR_1$, $-N=R$, $-OR$, $=O$, $-NO_2$, $-COOR$,

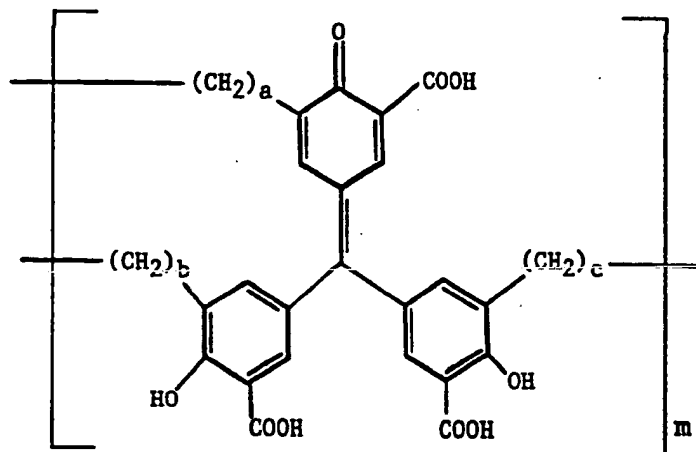
halogen, $-SO_2OR$, $-SO_2NHR$, $-OSO_2OR$ and $-R$;

R is lower alkyl or hydrogen; and

R_1 is lower alkyl, hydrogen phenyl or substituted phenyl; or

a pharmaceutically acceptable salt thereof.

12. A pharmaceutical composition according to Claim 11 wherein said polymer is of the formula



wherein a, b and c are 0 or 1 and $a+b+c \geq 2$ and m is about 5 to about 20; or a pharmaceutically acceptable salt thereof.

13. A pharmaceutical composition according to Claim 12 wherein the molecular weight of said polymer is about 2,000 to about 4,000 Daltons.

14. The pharmaceutical composition of Claim 13, wherein said polymer has anticoagulant properties and is capable of being absorbed into the bloodstream through the gastrointestinal tract.

15. A method of treating cardiovascular disorders comprising the administration to a human or other animal in need of such treatment a cardiovascular effective amount of a pharmaceutical composition according to Claim 1.

16. A method of treating metabolic disorders of bone tissue comprising the administration to a human or other animal in need of such treatment an effective bone metabolic amount of a pharmaceutical composition according to Claim 1.

17. A method of treating neuronal disorders comprising the administration to a human or other animal in need of such treatment an effective neuronal amount of a pharmaceutical composition according to Claim 1.

18. A method for affecting tissue redistribution of bioactive peptides and proteins which are normally bound to glycosaminoglycans comprising the administration of a pharmaceutical composition containing a tissue redistribution effective amount of a polymeric compound having a molecular weight of about 2,000 to about 20,000 Daltons and capable of mimicking the action of glycosaminoglycans in biological systems.